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Serum Creatinine and the DuPont Dimension: Possible Improvements

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Summary: A study is reported on the determination of creatinine with the DuPont Dimension, paying special attention to interference by bilirubin. Various methods for dealing with bilirubin interference are described, including modification of the *Jaffé* reaction, enzymatic assay of creatinine, enzymatic destruction of bilirubin, and ultrafiltration of the serum sample. None of these modifications gave completely satisfactory results, although the *Jaffé* method with a ferricyanide oxidation step, and the enzymatic assay with ferrocyanide gave reasonable results with icteric specimens from adult patients, while only the latter method could be used for neonatal serum samples.

Introduction

Recently we described our concern about the accuracy of the determination of creatinine in serum, especially in icteric specimens (1). We also mentioned the cooperation of the Bayer-Technicon company and the DuPont company. The cooperation with the first, needed because the Chem-1 is a closed system, resulted in preliminary data (2). Regarding the cooperation with DuPont, we were able to work more independently because of the open channel facility in the Dimension. Therefore we decided to continue the Dimension study by evaluating some modifications of creatinine methods described in the literature to circumvent or at least diminish the influence of bilirubin.

We tested the following approaches:

1. A kinetic *Jaffé* method incorporating a ferricyanide oxidation step to minimise the bilirubin inferference (3).
2. A kinetic *Jaffé* method including a pre-incubation with bilirubin oxidase (EC 1.3.3.5) (4).
3. An enzymatic procedure employing creatininase (EC 3.5.2.10) and creatinase (EC 3.5.3.3) as evaluated by *Guder* et al. (5) with the inclusion of potassium ferrocyanide to minimise the interference from bilirubin in the measurement of hydrogen peroxide (6).
4. The creatininase/creatinase procedure (as described under 3), but with pre-incubation with bilirubin oxidase instead of inclusion of potassium ferrocyanide (7).
5. Ultrafiltration of the serum sample and measurement of the ultrafiltrate with the current Dimension procedure which is a kinetic *Jaffé* method (8). DuPont provided an experimental Flex™ reagent cartridge, which they are developing for the ferricyanide oxidation step. They also provided preliminary software. The other approaches were studied with empty Flex™ cartridges, using our own reagents.

Materials and Methods

Materials

Creatinine, unconjugated bilirubin and potassium ferrocyanide ($K_4Fe(CN)_6$) were from Merck (Germany); conjugated bilirubin (ditaurate, di Na) from Porphyrin Products Inc. (Logan, Utah, U. S. A.); bilirubin oxidase (EC 1.3.3.5) from Sigma (St. Louis, U. S. A.) and human albumin from Behring Werke (Germany).

Methods

1. A special creatinine Flex™ cartridge, called XCREA, and containing potassium ferricyanide ($K_3Fe(CN)_6$), was prepared by DuPont for addition to the NaOH reagent wells. A separate software pad was developed for initiation of the reaction cycle.

2. The pre-incubation with bilirubin oxidase was performed for 5 minutes, according to the instructions in the product sheet.
3. Creatinine was determined kinetically with the Test-Combination, Creatinine PAP from Boehringer Mannheim (cat. No. 839434). Extra potassium ferrocyanide was included in the buffer/chromogen reagent: 8 mg $K_4Fe(CN)_6$ per 20 ml buffer/chromogen solution.
4. Ultrafiltration was performed with the Centrisart-I system from Sartorius (Germany), using a centrifuge with a swing-head rotor (10–15 min at 2900 *g*).
5. Interference experiments were done as described previously (1).
6. Reference mathod: see *Zwang* et al. (11).

Instrumentation

The DuPont Dimension (DuPont, U.S.A.) was operated according to the manufacturer's instructions, except that the calibration was based on the values obtained for patient samples run beforehand in the laboratory routine (Chem-1). This calibration procedure is standard in our laboratory though not recommended by DuPont. However, the differencies between both procedures are very small in our experience: 3–4% at elevated creatinine concentrations.

Patient samples

An elevated total bilirubin concentration was the only inclusion criterion for the study. A total of 175 serum samples was analysed in the first part. For the distribution of the bilirubin concentrations see table 1.

Tab. 1. Frequency distribution of the total bilirubin concentrations

Concentration range (µmol/l)	Number of samples
101–150	20
151–200	42
201–250	27
251–300	14
301–350	18
351–400	14
401–450	12
451–500	8
>500	20

The sera were stored at −80 °C until analysis (up to 4–6 weeks).

All except four of the analysed neonatal sera (n = 28) were pooled sera. They were also stored at −80 °C until analysis (up to 10–12 months).

Results

We started the study by analysing human albumin-containing creatinine standard solutions enriched with unconjucated bilirubin (1). This was done at three creatinine concentrations: ± 100, ± 300 and ± 500 µmol/l. In figure 1 only the results of the lowest concentration are depicted. The other two concentrations show a comparable picture.

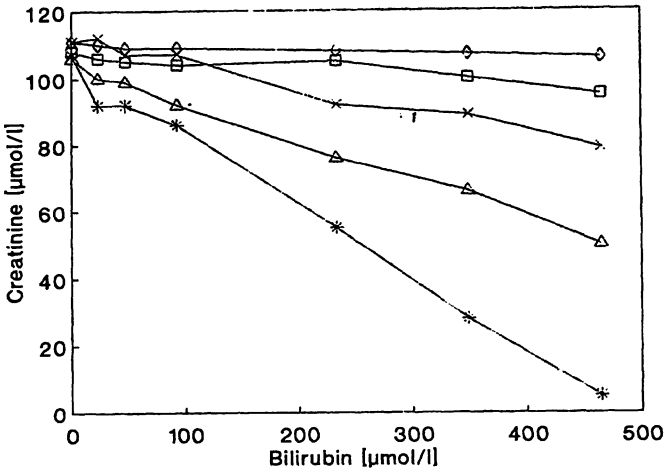

Fig. 1. The influence of unconjugated bilirubin (spiked human albumin solutions) on the various creatinine procedures.
◇ *Jaffé* including ferricyanide oxidation
□ *Jaffé* including pre-incubation with bilirubin oxidase
x Enzymatic including use of ferrocyanide
△ enzymatic including pre-incubation with bilirubin oxidase
* current method (*Jaffé*)

Experiments performed with conjugated bilirubin (ditaurobilirubin) revealed the same picture as shown in figure 1.

To test the practicability and accuracy of the results obtained with the creatinine standards, we continued the patient sample correlation study with methods 1 and 3 (see Introduction) in conjunction with the current Dimension procedure. Because all three methods were calibrated by comparison with our routine creatinine method, we first analysed 40 non-icteric, non-haemolytic and non-lipaemic serum samples with creatinine concentrations between 50 and 1200 µmol/l (equally distributed) by HPLC as a linearity check. The regression lines appeared to be nearly equivalent to the line y = x (slopes ± 3%, intercept 2–10 µmol/l).

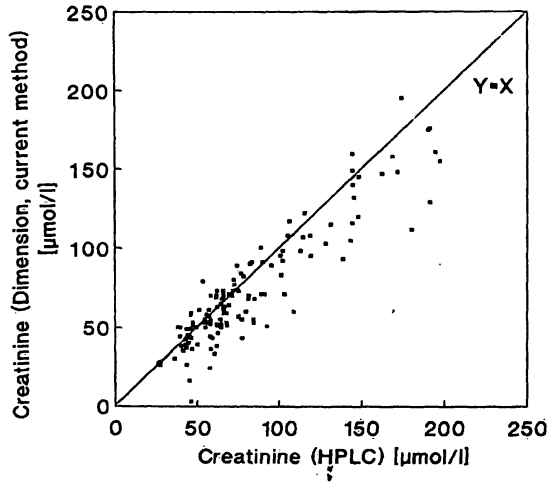

Fig. 2. Comparison of the current Dimension creatinine formulation (y-axis) and our HPLC-based comparison method (x-axis).

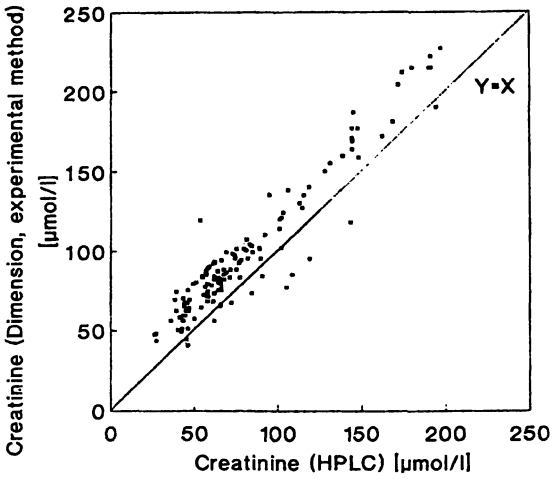

Fig. 3. Comparison of the experimental Dimension creatinine formulation (y-axis) and our HPLC-based comparison method (x-axis).

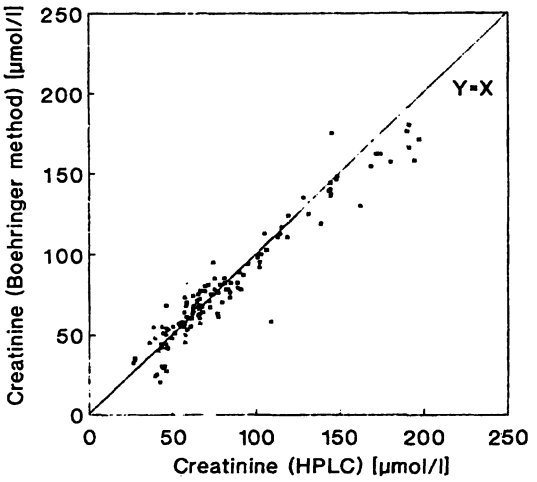

Fig. 4. Comparison of the modified enzymatic Boehringer creatinine procedure (y-axis) and our HPLC-based comparison (x-axis).

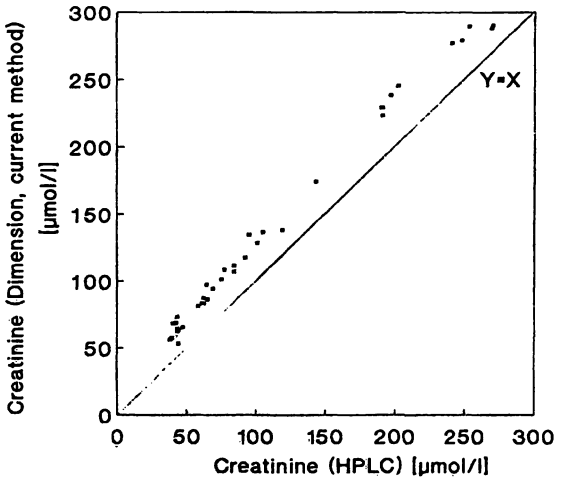

Fig. 5. Comparison of the results of the ultrafiltered icteric sera (current Dimension creatinine method) with those obtained with our HPLC-based comparison method (x-axis).
Statistical data (orthogonal regression procedure)
$y = 1.07x + 19.9$
$n = 37$; $r = 1.00$; $s_{y/x} = 4.1$ µmol/l

In figures 2, 3 and 4, comparison graphs are given of the measurements with 136 icteric serum samples. For clarity of presentation, we used a cut-off concentration for creatinine of 250 µmol/l.

Some of these specimens (n = 38) were also measured after ultrafiltration using aqueous creatinine standards. The results are plotted in figure 5.

Finally we analysed 28 neonatal icteric serum samples. The graphs of these comparisons are shown in figures 6, 7 and 8.

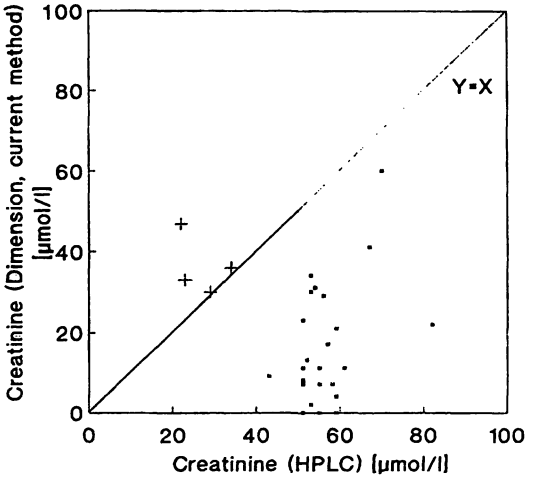

Fig. 6. Comparison of the current Dimension creatinine method (y-axis) and our HPLC-based comparison method (x-axis) for 28 neonatal samples (+ = fresh sample).

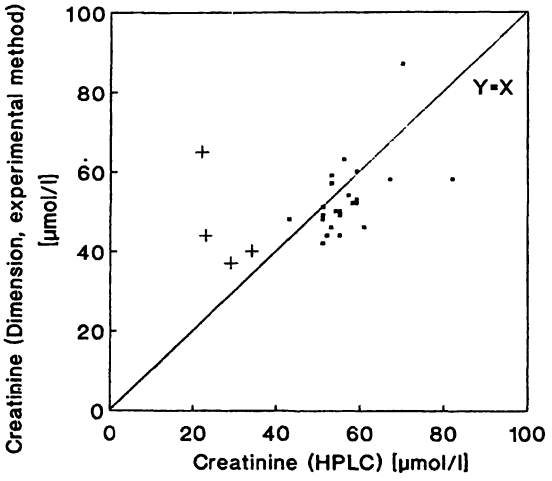

Fig. 7. Comparison of the experimental Dimension creatinine method (y-axis) and our HPLC-based comparison method (x-axis) for 28 neonatal samples (+ = fresh sample).

Discussion

It is clear from figures 1 to 4 that both modifications, the adapted enzymatic procedure and the experimental kinetic *Jaffé* method (XCREA), are improvements over the current Dimension formulation. Also, as

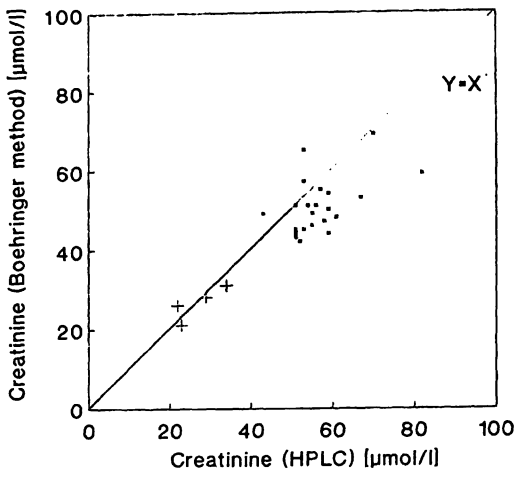

Fig. 8. Comparison of the modified enzymatic Boehringer creatinine procedure (y-axis) and our HPLC-based comparison method (x-axis) for 28 neonatal samples (+ = fresh sample).

Tab. 2. Statistical data of the various modifications
Method: orthogonal *Deming* procedure
The modification numbers refer to those described in the Introduction

Modification	Range (µmol/l)	n	r	$s_{y/x}$
Current *Jaffé*	0–1000	175	1.00	12.6
Revised *Jaffé* (mod. 1)	0–1000	175	1.00	12.1
Enzymatic (mod. 3)	0–1000	175	1.00	10.0
Current *Jaffé*	0–250	136	0.92	11.0
Revised *Jaffé* (mod. 1)	0–250	136	0.95	9.0
Enzymatic (mod. 3)	0–250	136	0.97	7.3
Ultrafiltration (mod. 5)	0–300	37	1.00	4.1

judged from the statistical parameters, the correlation coefficient r and the random error $s_{y/x}$ (see tab. 2), both of these described modifications for dealing with bilirubin interference appear to have a positive influence on the accuracy of the average result. On the other hand, plotting all data in a different way i.e. the residual creatinine results versus the bilirubin concentration, as in figures 9, 10 and 11, the overall impression seems to be more decisive.

Outlying residual results obtained with the enzymatic method can be understood for most of these results (creatinine concentrations not given), either because the creatinine concentrations are high to very high (> 500 µmol/l) and therefore the residuals less marked, or the interference clearing system is perhaps insufficient because of the very high bilirubin concentrations (> 300 µmol/l).

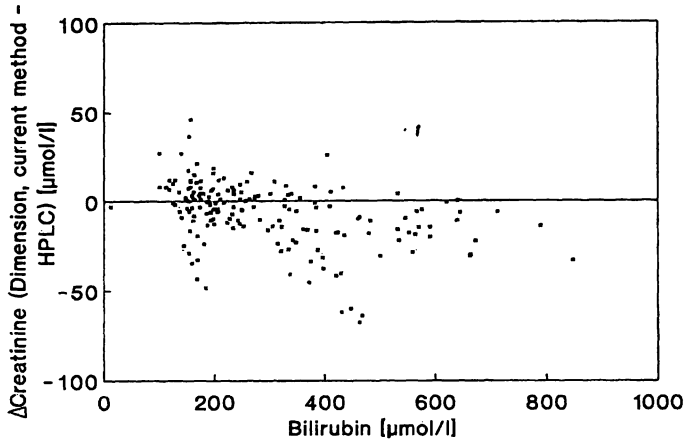

Fig. 9. Residual creatinine values (current Dimension minus HPLC) plotted against the total bilirubin results (x-axis).

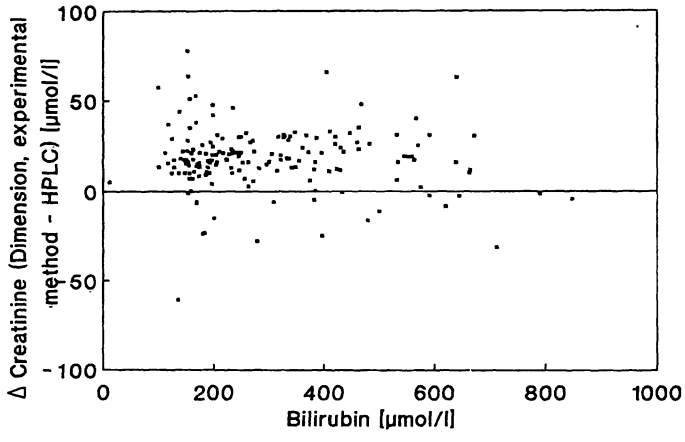

Fig. 10. Residual creatinine values (experimental Dimension minus HPLC) plotted against the total bilirubin results (x-axis).

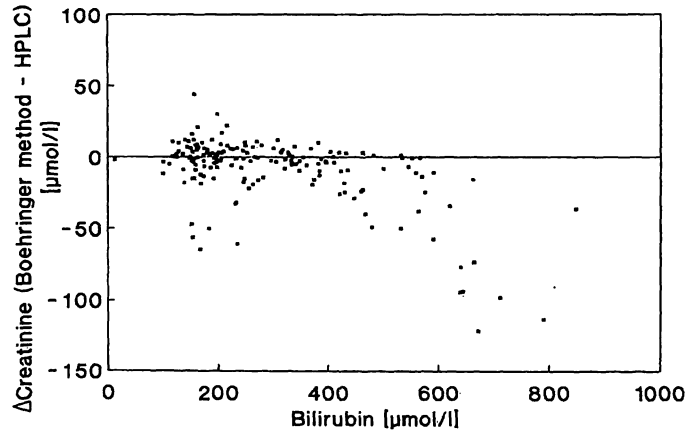

Fig. 11. Residual creatinine values (modified Boehringer minus HPLC) plotted against the total bilirubin results (x-axis).

For the other methods i.e. the current and the experimental *Jaffé* procedure (XCREA), the correlation between the residual creatinine values and the bilirubin concentrations is less obvious. This makes the improvement obtained with the experimental method

more difficult to understand, except by assuming that other interfering substances also play a role (9). Furthermore, we want to emphasize that we used a prototype Flex, which, in view of the present results, will probably not be the final configuration.

We plan to extend this part of the study, not only because we want to collect more patient-oriented information, but in order to increase the simplicity and practicability of the method.

For reasons mentioned earlier we decided not to extend the experiments with bilirubin oxidase. In addition, this preparation had a limited stability in our hands, which made the whole procedure very expensive. Possibly a different bilirubin oxidase procedure would lead to better results and a more practical procedure.

The interpretation of figures 6, 7 and 8 causes a different problem. It is clear that the enzymatic procedure has the best performance while the current method is the worst. However, there seems to be a difference in behaviour between the fresh and the stored samples, especially in the two *Jaffé* techniques. The fresh samples (marked +) though limited in number, form an exception. The other samples, some of them already brownish in colour, correlate less or even badly with HPLC, especially in the current procedure. Although we know that the storage conditions were in order, we may have asked too much i. e. four measurements with samples that are difficult to obtain.

Consideration of the figures requires care, not only because of the limited number of samples but also because of the range of creatinine values which may magnify any apparent discrepancy. On the other hand, this is what is normally seen with neonates: high bilirubin values and low creatinine.

We conclude that it makes sense to pay more attention to the enzymatic approach, not only because of the results we obtained (see figs. 4 and 8), which are satisfying but not completely satisfactory, but also for practical reasons. The stability of the reagent we used is very limited (only one day) which makes the method very expensive on the Dimension. There are other modifications on the market which may be worth testing with or without modification (10).

Our final remark concerns figure 5. The approach of ultrafiltration has understandably the advantage of enhanced accuracy. Of course, by applying the right calibration procedure, the results obtained with this approach can be equal to those with HPLC. On the other hand, the method is more time consuming and more expensive. The analytical system has the facility for applying the ultrafiltration method, and the decision is in the hands of the user.

Acknowledgement

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